Pyrogenic molecular markers: Linking PAH with BPCA analysis

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Abstract: Molecular characterization of pyrogenic organic matter (PyOM) is of great interest to understand the formation and behavior of these increasingly abundant materials in the environment. Two molecular marker methods have often been used to characterize and trace PyOM: polycyclic aromatic hydrocarbon (PAH) and benzenepolycarboxylic acid (BPCA) analysis. Since both methods target pyrogenic polycyclic compounds, we investigated the linkages between the two approaches using chars that were produced under controlled conditions. Rye and maize straws and their analogues charred at 300, 400 and 500 °C, respectively, were thus analyzed with both methods. Moreover, we also measured BPCAs directly on the lipid extracts, on which PAHs were analyzed, and on the respective extraction residues, too. Both methods revealed important features of the chars, in particular the increasing degree of aromatic condensation with increasing highest heating temperature (HTT). The overlap between the two methods was identified in the lipid fraction, where the proportion of benzenetricarboxylic acids (B3CAs) correlated with PAH abundance. The results confirmed the validity and complementarity of the two molecular marker methods, which will likely continue to play a crucial role in PyOM research due to the recent developments of compound-specific PAH and BPCA stable carbon ($d^{13}C$) and radiocarbon (^{1} C) isotope methods.

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Pyrogenic molecular markers: Linking PAH with BPCA analysis

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Abstract

Molecular characterization of pyrogenic organic matter (PyOM) is of great interest to understand the formation and behavior of these increasingly abundant materials in the environment. Two molecular marker methods have often been used to characterize and trace PyOM: polycyclic aromatic hydrocarbon (PAH) and benzenepolycarboxylic acid (BPCA) analysis. Since both methods target pyrogenic polycyclic compounds, we investigated the linkages between the two approaches using chars that were produced under controlled conditions. Rye and maize straws and their analogues charred at 300, 400 and 500 °C, respectively, were thus analyzed with both methods. Moreover, we also measured BPCAs directly on the lipid extracts, on which PAHs were analyzed, and on the respective extraction residues, too. Both methods revealed important features of the chars, in particular the increasing degree of aromatic condensation with increasing highest heating temperature (HTT). The overlap between the two methods was identified in the lipid fraction, where the proportion of benzenetricarboxylic acids (B3CAs) correlated with PAH abundance. The results confirmed the validity and complementarity of the two molecular marker methods, which will likely continue to play a crucial role in PyOM research due to the recent developments of compound-specific PAH and BPCA stable carbon ($\delta^{13}C$) and radiocarbon (^{14}C) isotope methods.

Keywords

Polycyclic aromatic hydrocarbons; benzene polycarboxylic acids; aromatic condensation; black carbon; biochar; pyrolysis temperature

Highlights

- PAH and BPCA marker methods for pyrogenic compounds were compared using chars.
- Both methods show increasing aromatic condensation of high temperature chars.
- Relationships between the two methods were found in the lipid fraction of the chars.
- The findings confirm the validity and theoretical assumptions of both methods.
- The methods complement each other in pyrogenic carbon research.

1. Introduction

Pyrogenic organic matter (PyOM) is ubiquitous in the environment (Schmidt and Noack, 2000; Schmidt et al., 2001; Masiello, 2004) and relatively stable against degradation, making it part of the slow-cycling C pool (Marschner et al., 2008; Kuzyakov et al., 2014). PyOM abundance is thought to increase globally with increasing wildfire occurrence in the 21st century (Flannigan et al., 2013; Kelly et al., 2013) and as a result of industrial combustion processes and traffic (Bond et al., 2013). Moreover, it is increasingly and intentionally produced in anthropogenic biochar-systems, which have been proposed as a strategy for green energy production, C sequestration, and soil improvement (Lehmann and Joseph, 2009; Meyer et al., 2011). With increasing PyOM production and the awareness of its importance for the C cycle, attention has been drawn to characterize these materials accurately across different disciplines (Simoneit, 2002; Hammes et al., 2007; McBeath et al., 2011). Molecular marker methods are one technique providing the opportunity to characterize PyOM. They target specifically the molecules that are produced during the combustion processes. Two different approaches are often used that both focus on the polycyclic structures typical for pyrogenic materials: i) polycyclic aromatic hydrocarbon (PAH) analysis and ii) the benzenepolycarboxylic acid (BPCA) method.

PAHs are small polycyclic aromatic compounds that have a long history as tracers for combustion products (Simoneit et al., 1999; Simoneit, 2002; Denis et al., 2012). They proofed to be particularly useful for the source apportionment of combustion residues in soils and sediments, for example distinguishing between contributions of biomass burning and fossil fuel (Oros and Simoneit, 2001a, b; Yunker et al., 2002; Bucheli et al., 2004). High concentrations of PAHs are of environmental concern due to their mutagenic and carcinogenic properties (Boffetta et al., 1997; Wilcke, 2000). Thus, PAHs have recently also been heavily investigated in the context of biochar applications (Hale et al., 2012; Hilber et al., 2012; Keiluweit et al., 2012;

70 Oleszczuk et al., 2013; Quilliam et al., 2013). After extraction with the lipid fraction from bulk
71 PyOM (Colmsjö, 1998; Fabbri et al., 2013), they are routinely quantified with gas or liquid
72 chromatography.

73 BPCAs, on the other hand, present a workaround to quantify the abundant polycyclic PyOM
74 structures that are larger and more complex than PAHs. These more condensed aromatic C
75 phases cannot be quantified directly as polymers using chromatographic methods. In order to
76 analyze such pyrogenic structures, PyOM is digested with nitric acid under high temperature and
77 pressure, which breaks the polycyclic compounds down into individual BPCAs, amenable to gas
78 or liquid chromatography (Glaser et al., 1998; Brodowski et al., 2005; Wiedemeier et al., 2013).
79 Pyrogenic C is thus assessed on a molecular level and can be used to estimate PyOM
80 abundance in environmental compartments such as soils and sediments. The technique has
81 yielded valuable insights into the slow cycling of PyOM in the pedosphere (Hammes et al., 2008;
82 Rodionov et al., 2010) and has been helpful to illuminate the pathways of PyOM into
83 sedimentary systems, where PyOM accounts for a quantitatively important fraction of the carbon
84 sink (Guggenberger et al., 2008; Sánchez-García et al., 2013). The BPCA method additionally
85 reveals information about the aromaticity, aromatic condensation and charring temperature of
86 the analyzed PyOM when relative yields of benzenetri-, benzenetetra-, benzenepenta- and
87 benzenehexacarboxylic acids (B3-, B4-, B5- and B6CA) are compared (Dittmar, 2008; Schneider
88 et al., 2011; Wiedemeier et al., 2014). These qualitative parameters are indicative for the stability
89 of PyOM in the environment and can also help to produce suitably engineered biochars (Harvey
90 et al., 2012a).

91 In this study, we investigated the link between the two molecular marker approaches. Despite
92 having different analysis windows, both methods target a part of the characteristic PyOM
93 polycyclic aromatic features that become increasingly condensed with higher charring
94 temperatures (McBeath et al., 2011). The aim was to test if the conceptually feasible overlap

between the small aromatic PAH moieties and the larger, condensed polycyclic structures, as indicated by BPCAs, can be assessed with the two methods when applied to real pyrogenic sample materials. We thus measured PAHs and BPCAs on two different straws (rye and maize) and their corresponding chars with highest heating temperatures (HTT) of 300, 400 and 500 °C, respectively. Moreover, we also analyzed BPCAs on the same lipid extracts, on which PAHs were measured, and determined BPCAs on the resulting extraction residues, too.

2. Materials and methods

Chars were produced by heating rye *(Secale cereal* L.) and maize (*Zea mays* L.) straw samples for 2 hours in a pre-heated muffle furnace at 300, 400 and 500 °C, respectively (Rennert et al., 2008). Al foil was used to limit oxidation during the charring process. Straw and char samples were subsequently milled to fine powder before chemical analysis.

Organic carbon contents were measured using elemental analysis (Leco CS 225, Mönchengladbach Germany). Lipids from all samples were extracted using the accelerated solvent extraction method (Dionex ASE 200, Sunnyvale, CA) with CH_2Cl_2/CH_3OH (93/7; v/v) as described by Wiesenberg et al. (2004; 2008; 2009). Samples were sequentially extracted for 20 min in two steps at 5×10^6 Pa and 75 °C and 140 °C, respectively. Both extracts were combined thereafter and cleaned using solid phase extraction columns fitted with KOH coated silica gel (Wiesenberg et al., 2008). Aromatic hydrocarbons were then measured after sequential chromatographic separation (Radke et al., 1980) on GC/MS (HP 5890 Series II GC and HP 5989A mass spectrometer, Palo Alto, CA). Detailed data from the PAH analysis of the samples were reported by Wiesenberg et al. (2009), while we here used specific statistics of these results with the focus to assess the linkages between the PAH and BPCA method.

BPCAs were measured on bulk materials (the two straws and all their charred analogues) as well as on the respective products from the lipid analysis (the lipid extracts and the lipid extraction residues). The lipid extracts of the 500 °C chars did not yield enough material to be amenable for BPCA analysis. BPCAs were analyzed following the procedure reported by Brodowski et al. (2005), i.e. bulk samples, lipid extracts and lipid extraction residues all underwent the same digestion and analysis steps. Method-inherent underestimation of PyOM was not corrected by any conversion factor (Schneider et al., 2010). For consistency purposes, we used gas chromatography protocols for both PAH and BPCA quantification. However, both

methods have recently been adapted to liquid chromatography, which offers an interesting alternative for the assessment of these markers (Schneider et al., 2011), particularly in environmental samples (Denis et al., 2012; Wiedemeier et al., 2013).

Data analysis was conducted with the statistical software R (R, 2011). Changes in the distribution of individual BPCAs between the bulk samples, the lipid extracts and the lipid extraction residues were analyzed with the chi-square goodness of fit test. Relationships between BPCAs and PAHs were analyzed with linear regression.

3. Results and discussion

PAHs were undetectable in the straw materials, present in chars with HTT of 300 °C and highly abundant in chars that were pyrolyzed between 400 and 500 °C (Figure 1; top). This is in line with previous studies (Brown et al., 2006; Kloss et al., 2012) and indicates the potentially problematic use of low-temperature biochars as a soil amendment due to their usually high content of harmful PAHs (Keiluweit et al., 2012). Moreover, a distinct pattern between the proportion of PAHs with different ring sizes and the pyrolysis temperature was observed: The proportion of small PAHs (3-ring) decreased while the contribution of higher molecular weight PAHs (4-6 ring) increased with increasing temperature (Figure 1; bottom). Similar trends have been observed in other studies (McGrath et al., 2003; Brown et al., 2006; Keiluweit et al., 2012) and point to the increasingly condensed nature of the solid PyOM residue (the char) while losses of the smaller, more volatile PAHs occur with increasing charring temperature (McGrath et al., 2003).

The BPCA method detects these large, condensed polycyclic structures of non-volatile PyOM and consequently showed increasing amounts of BPCA quantity with increasing temperature in the bulk char samples (Figure 2; black bars). Besides the total BPCA yields normalized to bulk weight, also the proportion of BPCA normalized to organic carbon increased with temperature. The latter is a measure for the aromaticity of charred materials (McBeath et al., 2011) and is consistently higher for rye-derived chars than for the maize-based char samples (Figure 2; upper number). This difference in aromaticity was partially reflected by the data obtained from the PAH analysis, as the rye chars showed higher proportions of the larger PAHs than the maize chars (Figure 1). However, the relationship between PAH ring sizes and aromaticity of PyOM are complex and not entirely resolved yet (Wiedemeier et al., 2014). The BPCA analysis furthermore yields a measure for the degree of aromatic condensation with the ratio of B6CAs to total BPCA

158 content (Schneider et al., 2011). The aromatic condensation was very similar for both, maize
159 and rye chars, at each respective charring temperature and comparable to previously reported
160 values for a variety of charred materials (Schneider et al., 2011; Wiedemeier et al., 2014). It
161 indicates that temperature is the main factor controlling condensation and that an assessment of
162 the aromatic condensation in PyOM is possibly useful to estimate its pyrolysis temperature.
163 Moreover, the degree of aromatic condensation in PyOM has been directly associated to its
164 stability against degradation in the environment (Harvey et al., 2012b; Wurster et al., 2013; Fang
165 et al., 2014). Our results in combination with complementary research (Wiedemeier et al., 2014)
166 therefore support the assumption that the C sequestration potential of charred lignocellulose
167 material is largely determined by its pyrolysis temperature (Bruun et al., 2011; Fang et al., 2014).

168 When lipid extraction was applied on the bulk straw and char samples, a part of the polycyclic
169 PyOM structures was extracted with it, too, while another part remained in the extraction
170 residue, as shown by the BPCA analysis in Figure 2 (white and striped bars, respectively). The
171 lipid extract always comprised a much smaller part (< 10 % of bulk dry weight) than the
172 extraction residue and became smaller for chars pyrolyzed at higher temperatures, following a
173 trend, which has been reported for other chars, too (Wiesenberg et al., 2009; Wiedemeier et al.,
174 2014). However, the proportion of lipid extracted PyOM increased with charring temperature
175 (Figure 2; white bars). This is explained by the increased availability of these polycyclic
176 structures and the concomitant decrease of other lipids with higher HTT (Wiesenberg and
177 Brodowski, 2007; Wiesenberg et al., 2009). At the same time, the much larger (> 90 % of bulk
178 dry weight) lipid extraction residue comprised a similar proportion of BPCA-detectable PyOM as
179 the lipid extract, also increasing with temperature (Figure 2; striped bars).

180 Having a closer look at the contributions of the individual BPCAs that make up the above
181 reported BPCA contents can reveal further qualitative information about the PyOM found in bulk
182 samples, lipid extracts and lipid extraction residues (Figure 3). In particular, the size of the PyOM

183 polycyclic clusters can be inferred, as the more carboxylated B6CA and B5CA must derive from
184 larger polycyclic clusters than the less carboxylated B4- and B3CAs (Hammes et al., 2008;
185 Schneider et al., 2010). Since the extraction residue inherited most of the bulk PyOM by mass (>
186 90 % of bulk dry weight), it is not surprising that it showed almost the same BPCA pattern like
187 the bulk chars, dominated by B6- and B5CAs (Figure 3). Bulk chars and the lipid extraction
188 residues thus mostly consisted of relatively large, condensed polycyclic sheets, comprising
189 probably the most stable aromatic phases of the chars (Franklin, 1951; Cohen-Ofri et al., 2006;
190 Keiluweit et al., 2010). In contrast, the lipid extractable BPCAs of both rye and maize chars
191 across all pyrolysis temperatures were mostly composed of smaller polycyclic compounds, as
192 indicated by the predominance of B3- and B4CAs. The larger polycyclic clusters that prevailed in
193 the bulk samples (57 – 67 % of B5- and B6CA) thus remained in the extraction residue (57 – 64
194 % of B5- and B6CA) while small polycyclic compounds were preferentially released during the
195 lipid extraction (51 – 59 % of B3- and B4CA).

196 The overlap between the PAH and the BPCA method consequently concerns mostly the
197 smallest BPCA-detectable polycyclic clusters. When BPCA analysis was performed on the lipid
198 extracts for direct comparison, we found significant logarithmic relationships ($p < 0.05$, $R^2 > 0.9$)
199 between the proportion of B3CAs and the abundance of most PAHs that were considered in this
200 study (Figure 4). The relationship was independent of the molecular weight of the PAHs and was
201 equally valid for total PAH abundance. However, the proportion of B3CA was the only direct
202 predictor of PAH abundance, while proportions of more carboxylated BPCAs in the lipid fraction
203 showed more complicated relationships with PAH abundance (cf. supplementary material). This
204 is in line with theoretical considerations (Glaser et al., 1998; Dittmar, 2008), after which the
205 majority of the abundant small PAHs (cf. Figure 1) are prone to yield B3- or B4CAs and only less
206 abundant, larger PAH species, such as benzo[a]pyrene or larger condensed polycyclic sheets,
207 can be oxidized into more carboxylated BPCAs. It is unfortunate that the 500 °C chars did not
208 yield enough lipid extract for BPCA analysis, which is why the comparison of the two methods

could not be conducted directly on the lipid extracts for the highest HTT of this study. However, as condensation of bulk chars increased (cf. Figure 2) but extractability of B5- and B6CA decreased with higher temperature (cf. Figure 3), a similar B3CA proportion can be expected in the 500 °C as in the 400 °C lipid extracts, correlating with the still high PAH abundances at 500 °C (cf. Figure 1). Our findings confirm that the analysis windows of the PAH and the BPCA method overlap each other within the lipid fraction. Although the linkage between the two molecular marker methods has been shown for pure chemical standards (Dittmar, 2008), to the best of our knowledge, it is the first time that the theory-based relationship was established empirically on real char samples. However, it is worth noting that no relationship could be established when both methods were applied directly on the bulk chars according to their normal protocol, as the BPCA analysis was then not limited to the lipid fraction only.

This study highlighted the similarities and differences of the two most common molecular marker methods for PyOM. Despite having minimally overlapping analysis windows and traditionally serving different research purposes, they are both highly informative for PyOM characterization as they provide valuable information about its molecular composition that is strongly linked to combustion conditions. We could demonstrate that the two molecular marker approaches find common ground when the lipid fraction is considered, which confirms the validity and theoretical assumptions of both methods. Large benefits can derive from the simultaneous application of the PAH and the BPCA method to relevant environmental sample materials. The combination of both methods can lead to a better source apportionment of PyOM in soils and sediments and illuminate the pathways and fluxes of differently sized and pyrolysed PyOM through environmental compartments. Even more information about PyOM in the environment can be retrieved from compound-specific isotopic analysis of both PAH and BPCA molecular markers (Ziolkowski and Druffel, 2010; Xu et al., 2012; Coppola et al., 2013; Slater et al., 2013; Gierga et al., 2014), which is why it is crucial to understand the here presented relationships between these two approaches.

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Figures

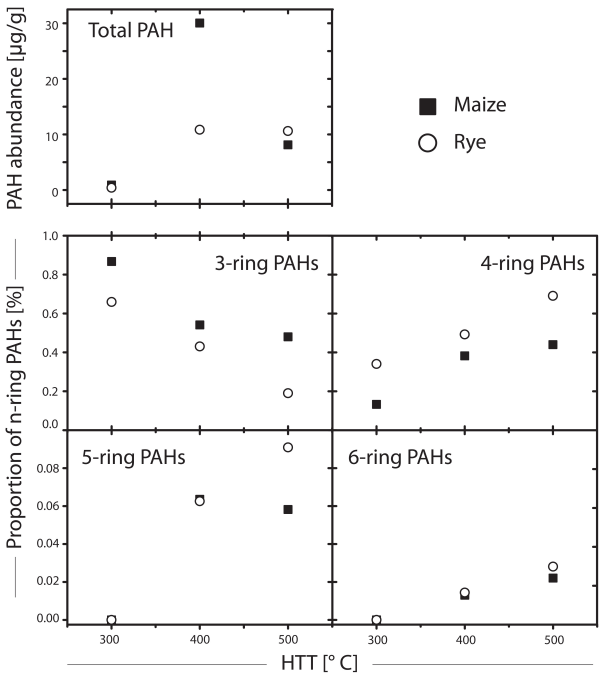

Figure 1.

Absolute (top) and relative (bottom) abundance of PAHs (n = 1) changed with highest heating temperature (HTT). Small, 3-ring PAHs were most abundant at HTT of 300 °C and decreased with higher HTT while 4-ring PAHs became more abundant with higher HTT. The larger PAHs (5- and 6-ring) contributed a much smaller proportion to total PAH (cf. y-axis scale is stretched by a factor of 10) and their abundance increased with higher HTT, consistent with an increasing degree of aromatic condensation as revealed by BPCAs (cf. Fig. 2).

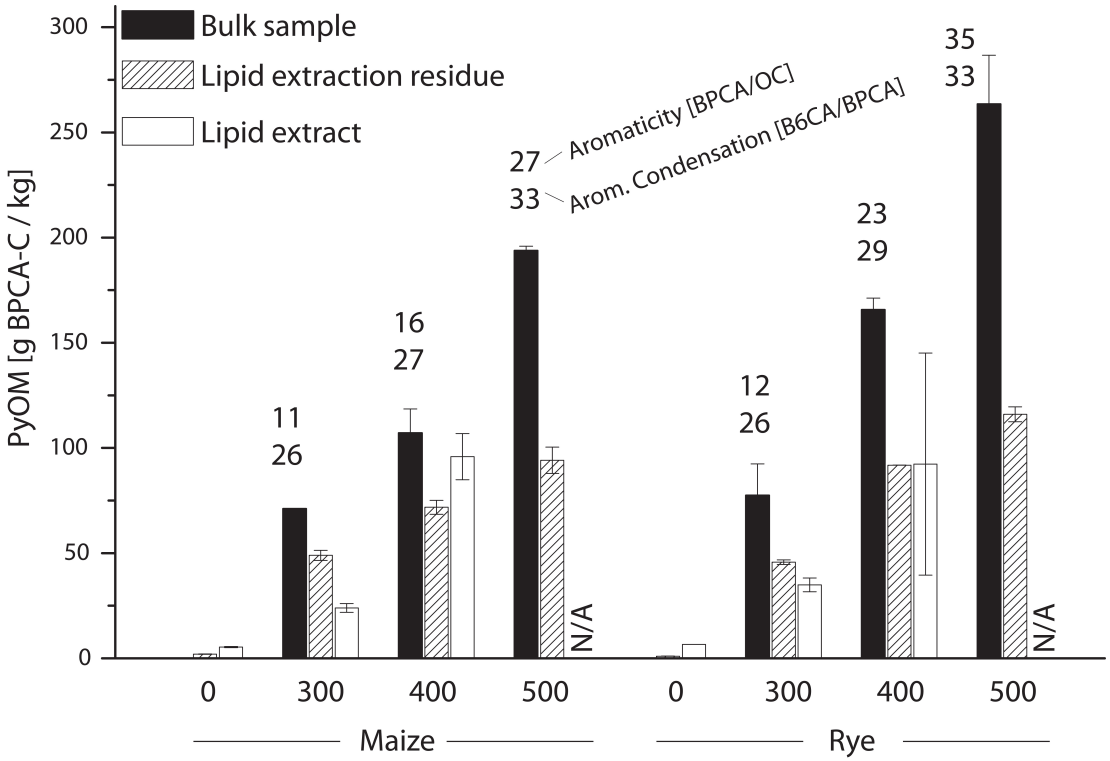

Figure 2.

PyOM measured as BPCAs in the bulk materials (maize/rye straw and chars), the respective lipid extracts and the remaining lipid extraction residues (n = 2 - 5, error bars indicate SE). The aromaticity and degree of aromatic condensation of the bulk samples increased with increasing HTT. Likewise, the PyOM content in the lipid extracts and in the lipid extraction residues increased with increasing HTT. The lipid extracts of the chars produced at 500 °C did not yield sufficient sample amounts for BPCA analysis.

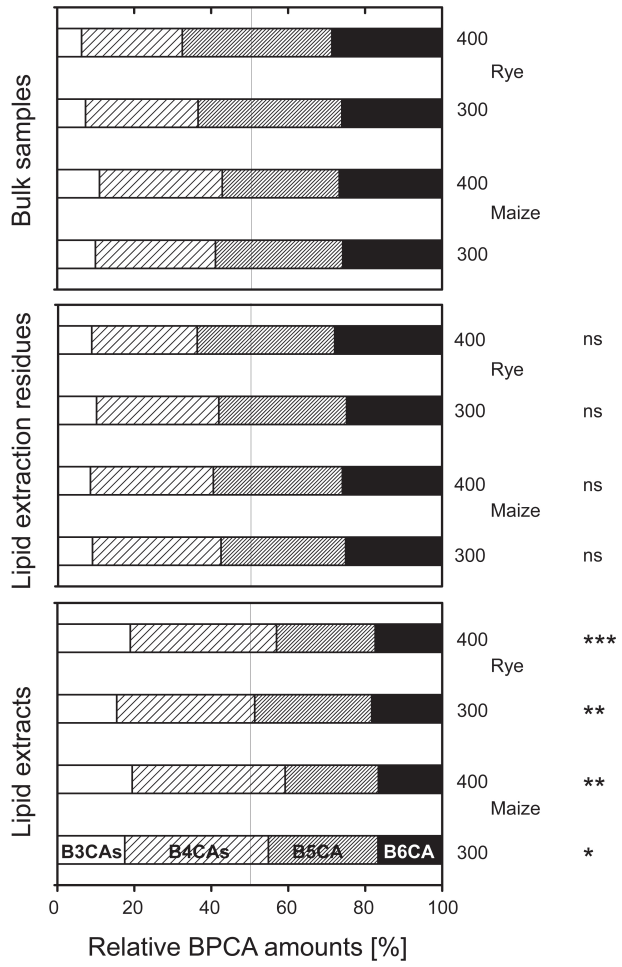

Figure 3.

BPCA signatures of bulk chars, their lipid extracts and the corresponding lipid extraction residues (n = 2 - 5, SE ≤ 3 percentage points). Larger PyOM polycyclic structures, indicated by B5CA and B6CA, were retained in the lipid extraction residues (ns = no significant difference in BPCA distribution between lipid extraction residue and respective bulk sample analogue). B3CA and B4CA, indicative of smaller polycyclic compounds, were preferentially extracted and accounted for more than 50 % of all BPCAs in the lipid extracts (asterisks denote significance of difference in BPCA distribution between lipid extract and respective bulk sample analogue with * $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$).

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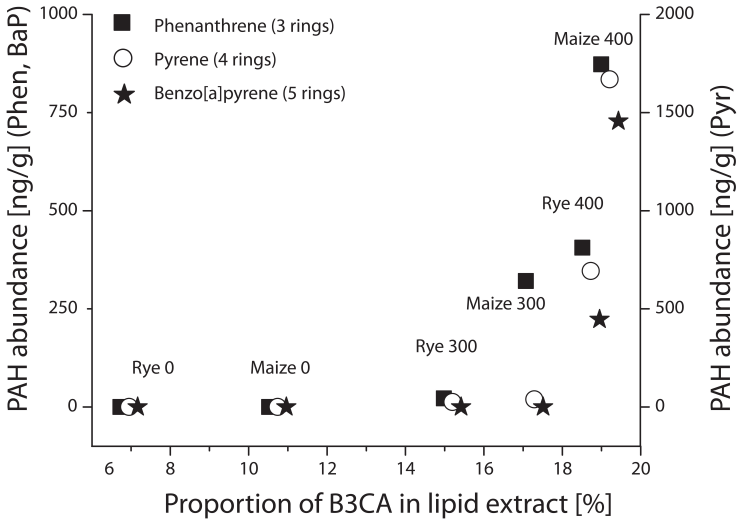

Figure 4.

The proportion of B3CA in the lipid extracts (n = 2, SE ≤ 1.4 percentage points) correlated with PAH abundance (n = 1). Data is shown for three different PAH sizes and is indicative for all PAHs considered in this study as well as for total PAH abundance. The correlation confirms that the measurement window of PAH analysis overlaps with B3CA analysis in the lipid extracts while no correlation could be found between PAHs and more carboxylated BPCAs, indicative of larger polycyclic clusters that would escape PAH analysis.

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